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(54) **PHOSPHODIESTERASE-NUCLEOTIDE CYCLIQUE HUMAINE**

(54) **HUMAN CYCLIC NUCLEOTIDE PDES**

(57)

<sup>22</sup> The invention provides human cyclic nucleotide phosphodiesterases (HSPDE10A)<sup>2</sup>and polynucleotides which identify and encode HSPDE10A. The invention also <sup>2</sup>provides<sup>2</sup>expression vectors host cells, antibodies, agonists, and antagonists. The <sup>2</sup>invention also<sup>2</sup>provides methods for diagnosing, treating, or preventing disorders associated <sup>2</sup>with expression<sup>2</sup>of HSPDE10A.<sup>2</sup>



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(57) **Abrégé/Abstract:**

The invention provides human cyclic nucleotide phosphodiesterases (HSPDE10A) and polynucleotides which identify and encode HSPDE10A. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HSPDE10A.

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## HUMAN CYCLIC NUCLEOTIDE PDES

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human cyclic nucleotide phosphodiesterases and to the use of these sequences in the diagnosis, treatment, and prevention of cancer and immune disorders.

### BACKGROUND OF THE INVENTION

Cyclic nucleotides (cAMP and cGMP) function as intracellular second messengers to transduce a variety of extracellular signals including hormones, light, and neurotransmitters. Cyclic nucleotide phosphodiesterases (PDEs) degrade cyclic nucleotides to the corresponding monophosphates, thereby regulating the intracellular concentrations of cyclic nucleotides and their effects on signal transduction. At least seven families of mammalian PDEs have been identified based on substrate specificity and affinity, sensitivity to cofactors, and sensitivity to inhibitory drugs (Beavo, J.A. (1995) *Physiol. Rev.* 75:725-748). Several of these families contain distinct genes, many of which are expressed in different tissues as splice variants. Within families, there are multiple isozymes and multiple splice variants of those isozymes. The existence of multiple PDE families, isozymes, and splice variants presents an opportunity for regulation of cyclic nucleotide levels and functions.

Type I PDEs (PDE1s) are  $\text{Ca}^{2+}$ /calmodulin-dependent and appear to be encoded by three different genes, each having at least two different splice variants. PDE1s have been found in the lung, heart, and brain. Some of the  $\text{Ca}^{2+}$ /calmodulin-dependent PDEs are regulated *in vitro* by phosphorylation/dephosphorylation. Phosphorylation of PDE1 decreases the affinity of the enzyme for calmodulin, decreases PDE activity, and increases steady state levels of cAMP. PDE2s are cGMP stimulated PDEs that are localized in the brain and are thought to mediate the effects of cAMP on catecholamine secretion. PDE3s are one of the major families of PDEs present in vascular smooth muscle. PDE3s are inhibited by cGMP, have high specificity for cAMP as a substrate, and play a role in cardiac function. One isozyme of PDE3 is regulated by one or more insulin-dependent kinases. PDE4s are the predominant isoenzymes in most inflammatory cells, and some PDE4s are activated by cAMP-dependent phosphorylation. PDE5s are thought to be cGMP specific but may also hydrolyze cAMP. High levels of PDE5s are found in most smooth muscle preparations, in platelets, and in the kidney. PDE6s play a role in vision and are regulated by light and cGMP. The PDE7 class, consisting of only one known member, is cAMP-specific and is most closely related to PDE4. PDE7 is not inhibited by rolipram, a specific inhibitor of PDE4 (Beavo, *supra*). PDE8 and PDE9 represent two newer families of PDEs. PDE8s are cAMP specific, most closely related to PDE4, insensitive to

rolipram, and sensitive to dipyridimole. PDE9s are cGMP specific and sensitive only to the PDE inhibitor zaprinast.

PDEs are composed of a catalytic domain of ~270 amino acids, an N-terminal regulatory domain responsible for binding cofactors, and, in some cases, a C-terminal domain of unknown function. A conserved motif, HDXXHXGXXN, has been identified in the catalytic domain of all PDEs. In PDE5, an N-terminal cGMP binding domain spans ~380 amino acid residues and comprises tandem repeats of the conserved sequence motif N(R/K)XnFX<sub>3</sub>DE (McAllister-Lucas, L.M. et al. (1993) J. Biol. Chem. 268:22863-22873). The NKXnD motif has been shown by mutagenesis to be important for cGMP binding (Turko, I.V. et al. (1996) J. Biol. Chem. 271:22240-22244). PDE families display approximately 30% amino acid identity within the catalytic domain; however, isozymes within the same family typically display about 85-95% identity in this region (e.g. PDE4A vs PDE4B). Furthermore, within a family there is extensive similarity (>60%) outside the catalytic domain; while across families, there is little or no sequence similarity.

Many functions of immune and inflammatory responses are inhibited by agents that increase intracellular levels of cAMP (Verghese, M.W. et al. (1995) Mol. Pharmacol. 47:1164-1171). A variety of diseases have been attributed to increased PDE activity and associated with decreased levels of cyclic nucleotides. A form of diabetes insipidus in the mouse has been associated with increased PDE4 activity, and an increase in low-K<sub>m</sub> cAMP PDE activity has been reported in leukocytes of atopic patients. Defects in PDEs have also been associated with retinal disease. Retinal degeneration in the rd mouse, autosomal recessive retinitis pigmentosa in humans, and rod/cone dysplasia I in Irish Setter dogs have been attributed to mutations in the PDE6B gene. PDE3 has been associated with cardiac disease.

Many inhibitors of PDEs have been identified and have undergone clinical evaluation. PDE3 inhibitors are being developed as antithrombotic agents, antihypertensive agents, and as cardiotonic agents useful in the treatment of congestive heart failure. Rolipram, a PDE4 inhibitor, has been used in the treatment of depression, and other inhibitors of PDE4 are undergoing evaluation as anti-inflammatory agents. Rolipram has also been shown to inhibit lipopolysaccharide (LPS) induced TNF- $\alpha$  which has been shown to enhance HIV-1 replication *in vitro*. Therefore, rolipram may inhibit HIV-1 replication (Angel, J.B. et al. (1995) AIDS 9:1137-1144). Additionally, rolipram, based on its ability to suppress the production of cytokines such as TNF- $\alpha$  and  $\beta$  and interferon  $\gamma$ , has been shown to be effective in the treatment of encephalomyelitis. Rolipram may also be effective in treating tardive dyskinesia and was effective in treating multiple sclerosis in an experimental animal model (Sommer, N. et al. (1995) Nat. Med. 1:244-248; Sasaki, H. et al. (1995) Eur. J. Pharmacol 282:71-76).

Theophylline is a nonspecific PDE inhibitor used in the treatment of bronchial asthma and

other respiratory diseases. Theophylline is believed to act on airway smooth muscle function and in an anti-inflammatory or immunomodulatory capacity in the treatment of respiratory diseases (Banner, K.H. and C.P. Page (1995) Eur. Respir. J. 8:996-1000). Pentoxifylline is another nonspecific PDE inhibitor used in the treatment of intermittent claudication and diabetes-induced peripheral vascular disease. Pentoxifylline is also known to block TNF- $\alpha$  production and may inhibit HIV-1 replication (Angel et al., supra).

PDEs have also been reported to effect cellular proliferation of a variety of cell types and have been implicated in various cancers. Bang et al. (1994; Proc. Natl. Acad. Sci. USA 91:5330-5334) reported that growth of prostate carcinoma cell lines DU 145 and LNCaP was inhibited by delivery of cAMP derivatives and phosphodiesterase inhibitors. These cells also showed a permanent conversion in phenotype from epithelial to neuronal morphology. Others have suggested that PDE inhibitors have the potential to regulate mesangial cell proliferation (Matousovic, K. et al. (1995) J. Clin. Invest. 96:401-410) and lymphocyte proliferation (Joulain, C. et al. (1995) J. Lipid Mediat. Cell Signal. 11:63-79). Finally, Deonarain and Epenetos (1994; Br. J. Cancer 70:786-94) describe a cancer treatment that involves intracellular delivery of phosphodiesterases to particular cellular compartments of tumors which results in cell death.

The discovery of new human cyclic nucleotide phosphodiesterases and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cancer and immune disorders.

## SUMMARY OF THE INVENTION

The invention features purified polypeptides, human cyclic nucleotide phosphodiesterases, referred to collectively as "HSPDE10A" and individually as "HSPDE10A1" and "HSPDE10A2." In one aspect, the invention provides an isolated polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-2.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino

acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:3-4.

5 Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid  
10 sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino  
15 acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is  
20 transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an  
25 amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

The invention further provides an isolated polynucleotide comprising a) a polynucleotide  
30 sequence selected from the group consisting of SEQ ID NO:3-4, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, c) a polynucleotide sequence complementary to a), or d) a polynucleotide sequence complementary to b). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

35 Additionally, the invention provides a method for detecting a target polynucleotide in a

sample, said target polynucleotide having a sequence of a polynucleotide comprising a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, c) a polynucleotide sequence complementary to a), or d) a polynucleotide sequence complementary to b). The method comprises a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 30 contiguous nucleotides. In another alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and a pharmaceutically acceptable excipient. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional HSPDE10A, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional HSPDE10A, comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising a) an amino acid sequence selected from the group



consisting of SEQ ID NO:1-2, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or d) an immunogenic fragment of an amino acid sequence selected from the group

5 consisting of SEQ ID NO:1-2. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional

10 HSPDE10A, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:3-4, the method comprising a) exposing a

15 sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

#### BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figures 1A, 1B, 1C, 1D, and 1E show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:3) of HSPDE10A1. The alignment was produced using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA).

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Figures 2A, 2B, 2C, 2D, 2E, and 2F show the amino acid sequence (SEQ ID NO:2) and nucleic acid sequence (SEQ ID NO:4) of HSPDE10A2. The alignment was produced using MACDNASIS PRO software (Hitachi Software Engineering).

Figures 3A, 3B, 3C, 3D, and 3E show the amino acid sequence alignments between HSPDE10A1 (SEQ ID NO:1), HSPDE10A2 (SEQ ID NO:2), and human PDE5, HPDE5A1 (GI 3355606; SEQ ID NO:5), produced using the MEGALIGN program (DNASTAR, Madison WI).

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Figures 4A and 4B show the activity assay for HSPDE10A1 using cAMP and cGMP as substrates, respectively. The positive X axis represents the substrate concentration ( $\mu\text{M}$ ), and the positive Y axis represents the reaction velocity in pmoles/minute/ml enzyme.  $K_m$  and  $V_{max}$  values for the enzyme activity with each substrate were calculated from a Michaelis-Menten plot using the "Fit Curve" Microsoft Excel extension program.

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Figures 5A and 5B show the membrane-based northern analysis of HSPDE10A expression in human tissues. The X axis presents the various tissues analyzed and the Y axis presents various size

markers. The arrow indicates the location of the major (~7.5 kb) transcript of HSPDE10A.

Figure 6 shows the expression of full length HSPDE10A1 in Sf9 cells (arrow; predicted molecular weight ~56 kDa). Lane 1 shows various size markers and their molecular weights. Lanes 2 and 4 show HSPDE10A1 in infected cells at 64,000 and 12,800 cell equivalents, respectively. Lanes 3 and 5, mock infected cells at 64,000 and 12,800 cell equivalents, respectively, fail to show the presence of HSPDE10A1.

Table 1 shows the effects of various PDE inhibitors on the activity of HSPDE10A1. Assays were carried out using cGMP as a substrate at a concentration of 0.17  $\mu$ M, equal to ~1/3 of the  $K_m$  of cGMP. Inhibitors were tested over a range of concentrations from ~0.5 to ~110  $\mu$ M.  $IC_{50}$  (or  $K_i$ ) values were extrapolated from the dose response curves.

Table 2 shows the tools, programs, and algorithms used to analyze HSPDE10A, along with applicable descriptions, references, and threshold parameters.

### DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

### DEFINITIONS

"HSPDE10A" refers to the amino acid sequences of substantially purified HSPDE10A

obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HSPDE10A. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HSPDE10A either by directly interacting with HSPDE10A or by acting on components of the biological pathway in which HSPDE10A participates.

An "allelic variant" is an alternative form of the gene encoding HSPDE10A. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HSPDE10A include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HSPDE10A or a polypeptide with at least one functional characteristic of HSPDE10A. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HSPDE10A, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HSPDE10A. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HSPDE10A. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HSPDE10A is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally

occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence.

- 5 Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of HSPDE10A. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HSPDE10A  
10 either by directly interacting with HSPDE10A or by acting on components of the biological pathway in which HSPDE10A participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HSPDE10A polypeptides can be prepared using intact polypeptides or using  
15 fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize  
20 the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures  
25 on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the  
30 complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the  
35 capability of the natural, recombinant, or synthetic HSPDE10A, or of any oligopeptide thereof, to

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induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HSPDE10A or fragments of HSPDE10A may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser

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	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
	His	Asn, Arg, Gln, Glu
5	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
10	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
15	Val	Ile, Leu, Thr

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Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HSPDE10A or the polynucleotide encoding HSPDE10A which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the

present embodiments.

A fragment of SEQ ID NO:3-4 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:3-4, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:3-4 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:3-4 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:3-4 and the region of SEQ ID NO:3-4 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-2 is encoded by a fragment of SEQ ID NO:3-4. A fragment of SEQ ID NO:1-2 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-2. For example, a fragment of SEQ ID NO:1-2 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-2. The precise length of a fragment of SEQ ID NO:1-2 and the region of SEQ ID NO:1-2 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in  
 5 Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

10 Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis  
 15 programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST  
 20 programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Reward for match: 1*

25 *Penalty for mismatch: -2*

*Open Gap: 5 and Extension Gap: 2 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 11*

30 *Filter: on*

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous  
 35 nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported



by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 3*

*Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be

suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of HSPDE10A. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HSPDE10A.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Probe" refers to nucleic acid sequences encoding HSPDE10A, their complements, or

fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule.

Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

“Primers” are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a “mispriming library,” in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user’s specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing

selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HSPDE10A, or fragments thereof, or HSPDE10A itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by

different amino acids or nucleotides, respectively.

“Substrate” refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

“Transformation” describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term “transformed” cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A “variant” of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the “BLAST 2 Sequences” tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an “allelic” (as defined above), “splice,” “species,” or “polymorphic” variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass “single nucleotide polymorphisms” (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A “variant” of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the “BLAST 2 Sequences” tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at

least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

### THE INVENTION

The invention is based on the discovery of new human cyclic nucleotide phosphodiesterases (HSPDE10A), the polynucleotides encoding HSPDE10A, and the use of these compositions for the diagnosis, treatment, or prevention of cancer and immune disorders.

Nucleic acids encoding the HSPDE10A of the present invention were identified in Incyte Clone 826776 from the prostate cDNA library (PROSTUT04) using BLAST analysis and human PDE5 (GI 3355606) as a query sequence against the LIFESEQ database (Incyte Pharmaceuticals, Palo Alto CA). Full length cDNA sequences of HSPDE10A were obtained from a human skeletal muscle library using the complete cDNA insert of Incyte Clone 826776 as a hybridization probe.

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. As shown in Figures 1A, 1B, 1C, 1D, and 1E, HSPDE10A1 is 490 amino acids in length and has a putative cGMP binding motif in the sequence N88RLDGKPFDDAD of SEQ ID NO:1 and a PDE signature motif at H260DLDRGTNN of SEQ ID NO:1. As shown in Figures 3A, 3B, 3C, 3D, and 3E, HSPDE10A1 has chemical and structural similarity with human PDE5, HSPDE5A1 (GI 3355606; SEQ ID NO:5). In particular, HSPDE10A1 and HSPDE5A1 share 42% identity. The ~270 amino acid catalytic domain found in all PDEs extends between approximately residues F196 and R458 in HSPDE10A1, and is 50% identical to HSPDE5A1 in this region. The putative cGMP binding motif in HSPDE10A1 beginning at residue N88 is coincident with the tandem repeat motif for cGMP binding in HSPDE5A1 beginning at residue N472, and the PDE signature sequence for HSPDE10A1 beginning at residue H260 is conserved in HSPDE5A as well. HSPDE10A1 shares a slightly lesser degree of homology, ranging from 25% to 44%, with other representatives of PDE families 1, 2, 3, 4, 6, 7, 8, and 9 (data not shown). The fragment of SEQ ID NO:3 from about nucleotide 1168 to about nucleotide 1212 is useful in hybridization or amplification technologies to identify SEQ ID NO:3 and to distinguish between SEQ ID NO:3 and a related sequence.

In another embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2. As shown in Figures 2A, 2B, 2C, 2D, and 2E, HSPDE10A2 is 367 amino acids in length, and also contains the putative cGMP binding motif at N88RLDGKPFDDAD of SEQ ID NO:2 and a PDE signature motif at H260DLDRGTNN of SEQ ID NO:2. As shown in Figures 3A, 3B, 3C, 3D, and 3E, HSPDE10A2 is identical to HSPDE10A1 between residues M1 and E338, but differs in the C-terminal portion of the molecule from E339 to Y367. HSPDE10A2 also shares 40% identity with HSPDE5A1. The fragment of SEQ ID NO:4 from about nucleotide 1183 to about nucleotide 1227 is useful in hybridization or amplification technologies to identify SEQ ID NO:4 and

to distinguish between SEQ ID NO:4 and a related sequence.

A cDNA construct encoding the full length amino acid sequence of HSPDE10A1 was cloned into the baculovirus transfer vector pFASTBAC, expressed in Sf9 cells, and the enzyme partially purified from these cells for enzyme assays. Figures 4A and 4B show the kinetics of HSPDE10A1 enzyme activity with cAMP (Figure 4A) and cGMP (Figure 4B) as substrates. Both substrates are hydrolyzed at a similar rate ( $V_{max} = 0.23$  and  $0.21 \mu\text{mole/min}/\mu\text{l}$  enzyme for cAMP and cGMP, respectively), and with a similar affinity for HSPDE10A1 ( $K_m = 1.04$  and  $0.52 \mu\text{M}$  for cAMP and cGMP, respectively). The data confirms that HSPDE10A1 is a PDE capable of hydrolyzing both cAMP and cGMP at physiologically relevant concentrations. The effects of various known PDE inhibitors on the activity of HSPDE10A1 using cGMP as a substrate are shown in Table 1. HSPDE10A1 was relatively insensitive to both milrinone and rolipram, which are selective for PDE3 and PDE4 respectively, with  $IC_{50}$  values of  $>200 \mu\text{M}$  and  $160 \mu\text{M}$ , respectively. The non-selective PDE inhibitor IBMX (3-isobutyl-1-methylxanthine) inhibited HSPDE10A1 with an  $IC_{50}$  of  $40 \mu\text{M}$ , which is within the range observed for other PDEs, except the IBMX-insensitive PDE8. The so-called cGMP PDE-specific inhibitor zaprinast, which is selective for PDE5 and PDE6, was moderately potent against HSPDE10A1 with an  $IC_{50}$  of  $8 \mu\text{M}$  (10-40 fold higher than PDEs 5 and 6).

The degree of similarity exhibited between the HSPDE10A1 and representatives of the other families of PDEs in the catalytic domain (25% to 50%) is consistent with that demonstrated between different PDE families (~30 %). HSPDE10A1 is further distinguished from other known families by its dual specificity for both cAMP and cGMP and by its pattern of inhibition by known PDE inhibitors. HSPDE10A1 therefore appears to be a member of a new family of cyclic nucleotide phosphodiesterases designated PDE10.

Membrane-based northern analysis (Figures 6A and 6B) shows the expression of HSPDE10A as a major transcript of ~7.5 kb in skeletal muscle and prostate tissue, with an additional ~3.0 kb mRNA detected in prostate alone. A less prominent transcript of ~1.5 kb occurs in testes and skeletal muscle as well. These data suggest that at least three HSPDE10A splice variants exist. Electronic northern analysis using the LIFESEQ database (Incyte Pharmaceuticals) further shows the expression of HSPDE10A in cancerous prostate tissue.

Figure 6 shows the expression of HSPDE10A1 in cell lysates of Sf9 cells transfected with a baculovirus vector containing an untagged cDNA construct. An approximately 56 kDa polypeptide could be detected either by Coomassie blue staining (native HSPDE10A1; Figure 6) or by western immunoblotting of a FLAG-tagged HSPDE10A1 using an anti-FLAG antibody (data not shown).

The invention also encompasses HSPDE10A variants. A preferred HSPDE10A variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino



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acid sequence identity to the HSPDE10A amino acid sequence, and which contains at least one functional or structural characteristic of HSPDE10A.

The invention also encompasses polynucleotides which encode HSPDE10A. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:3-4, which encodes HSPDE10A.

The invention also encompasses a variant of a polynucleotide sequence encoding HSPDE10A. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HSPDE10A. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:3-4 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:3-4. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HSPDE10A.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HSPDE10A, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HSPDE10A, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HSPDE10A and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HSPDE10A under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HSPDE10A or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HSPDE10A and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HSPDE10A and HSPDE10A derivatives, or fragments thereof, entirely by synthetic chemistry. After production,

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the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HSPDE10A or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of  
 5 hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:3-4 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

10 Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE  
 15 amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics,  
 20 Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HSPDE10A may be extended utilizing a partial  
 25 nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) *PCR Methods Applic.* 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to  
 30 amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119.) In this method, multiple  
 35 restriction enzyme digestions and ligations may be used to insert an engineered double-stranded

sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) *Nucleic Acids Res.* 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure  
5 avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

10 When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

15 Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate  
20 software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof  
25 which encode HSPDE10A may be cloned in recombinant DNA molecules that direct expression of HSPDE10A, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HSPDE10A.

The nucleotide sequences of the present invention can be engineered using methods generally  
30 known in the art in order to alter HSPDE10A-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction  
35 sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HSPDE10A may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.)

Alternatively, HSPDE10A itself or a fragment thereof may be synthesized using chemical methods.

- 5 For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HSPDE10A, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

- 10 The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

- 15 In order to express a biologically active HSPDE10A, the nucleotide sequences encoding HSPDE10A or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in
- 20 polynucleotide sequences encoding HSPDE10A. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HSPDE10A. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HSPDE10A and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no
- 25 additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular
- 30 host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

- Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HSPDE10A and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A
- 35 Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et

al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HSPDE10A. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HSPDE10A. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HSPDE10A can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPT1 plasmid (Life Technologies). Ligation of sequences encoding HSPDE10A into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for *in vitro* transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509.) When large quantities of HSPDE10A are needed, e.g. for the production of antibodies, vectors which direct high level expression of HSPDE10A may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HSPDE10A. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast *Saccharomyces cerevisiae* or *Pichia pastoris*. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, *supra*; Bitter, G.A. et al. (1987) *Methods Enzymol.* 153:516-544; and Scorer, C.A. et al. (1994) *Bio/Technology* 12:181-184.)

Plant systems may also be used for expression of HSPDE10A. Transcription of sequences encoding HSPDE10A may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; and Winter, J. et al. (1991) *Results Probl. Cell Differ.* 17:85-105.)

These constructs can be introduced into plant cells by direct DNA transformation or

pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HSPDE10A may be ligated  
 5 into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HSPDE10A in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.  
 10 SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet.  
 15 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HSPDE10A in cell lines is preferred. For example, sequences encoding HSPDE10A can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate  
 20 vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These  
 25 include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* and *apv* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to  
 30 methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc.  
 35 Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins

(GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) *Methods Mol. Biol.* 55:121-131.)

5        Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HSPDE10A is inserted within a marker gene sequence, transformed cells containing sequences encoding HSPDE10A can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HSPDE10A under  
10       the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

      In general, host cells that contain the nucleic acid sequence encoding HSPDE10A and that express HSPDE10A may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR  
15       amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

      Immunological methods for detecting and measuring the expression of HSPDE10A using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and  
20       fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HSPDE10A is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) *Serological Methods, a Laboratory Manual*, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) *Current Protocols in Immunology*, Greene Pub. Associates and  
25       Wiley-Interscience, New York NY; and Pound, J.D. (1998) *Immunochemical Protocols*, Humana Press, Totowa NJ.)

      A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HSPDE10A  
30       include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HSPDE10A, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted  
35       using a variety of commercially available kits, such as those provided by Amersham Pharmacia

Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HSPDE10A may be cultured  
5 under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HSPDE10A may be designed to contain signal sequences which direct secretion of HSPDE10A through a prokaryotic or eukaryotic cell membrane.

10 In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity.  
15 Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid  
20 sequences encoding HSPDE10A may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HSPDE10A protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HSPDE10A activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially  
25 available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity  
30 purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HSPDE10A encoding sequence and the heterologous protein sequence, so that HSPDE10A may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995,  
35 supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and



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purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HSPDE10A may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated  
5 with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, <sup>35</sup>S-methionine.

Fragments of HSPDE10A may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis  
10 may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HSPDE10A may be synthesized separately and then combined to produce the full length molecule.

#### THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between  
15 regions of HSPDE10A and human cyclic nucleotide phosphodiesterases. In addition, the expression of HSPDE10A is closely associated with skeletal muscle and with normal and cancerous prostate tissue. Therefore, HSPDE10A appears to play a role in cancer and immune disorders. In particular, inhibitors of PDE have been shown to be effective in the treatment of these types of diseases and disorders. In the treatment of disorders associated with increased HSPDE10A expression or activity,  
20 it is desirable to decrease the expression or activity of HSPDE10A. In the treatment of disorders associated with decreased HSPDE10A expression or activity, it is desirable to increase the expression or activity of HSPDE10A.

Therefore, in one embodiment, HSPDE10A or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or  
25 activity of HSPDE10A. Examples of such disorders include, but are not limited to, a cancer, such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an immune  
30 disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia  
35 with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis,

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glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic  
5 lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

In another embodiment, a vector capable of expressing HSPDE10A or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with  
10 decreased expression or activity of HSPDE10A including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HSPDE10A in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPDE10A including, but not limited to, those provided above.

15 In still another embodiment, an agonist which modulates the activity of HSPDE10A may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPDE10A including, but not limited to, those listed above.

In a further embodiment, an antagonist of HSPDE10A may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HSPDE10A. Examples  
20 of such disorders include, but are not limited to, those cancers and immune disorders described above. In one aspect, an antibody which specifically binds HSPDE10A may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HSPDE10A.

In an additional embodiment, a vector expressing the complement of the polynucleotide  
25 encoding HSPDE10A may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HSPDE10A including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made  
30 by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HSPDE10A may be produced using methods which are generally known in  
35 the art. In particular, purified HSPDE10A may be used to produce antibodies or to screen libraries of

pharmaceutical agents to identify those which specifically bind HSPDE10A. Antibodies to HSPDE10A may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HSPDE10A or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HSPDE10A have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HSPDE10A amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HSPDE10A may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HSPDE10A-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte

population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HSPDE10A may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HSPDE10A and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HSPDE10A epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HSPDE10A. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of HSPDE10A-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HSPDE10A epitopes, represents the average affinity, or avidity, of the antibodies for HSPDE10A. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular HSPDE10A epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the HSPDE10A-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HSPDE10A, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC; Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of

HSPDE10A-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, *supra*, and Coligan et al. *supra*.)

In another embodiment of the invention, the polynucleotides encoding HSPDE10A, or any  
5 fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HSPDE10A may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HSPDE10A. Thus, complementary molecules or fragments may be used to modulate HSPDE10A activity, or to achieve regulation of gene function.  
10 Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HSPDE10A.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted  
15 organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HSPDE10A. (See, e.g., Sambrook, *supra*; Ausubel, 1995, *supra*.)

Genes encoding HSPDE10A can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding  
20 HSPDE10A. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HSPDE10A. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing  
30 is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, *Molecular and Immunologic Approaches*, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block  
35 translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HSPDE10A.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding HSPDE10A. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and

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monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HSPDE10A, antibodies to HSPDE10A, and mimetics, agonists, antagonists, or inhibitors of HSPDE10A. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to

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characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or  
5 starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's  
10 solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic  
15 amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

20 The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic  
25 acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate  
30 container and labeled for treatment of an indicated condition. For administration of HSPDE10A, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

35 For any compound, the therapeutically effective dose can be estimated initially either in cell



culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

5       A therapeutically effective dose refers to that amount of active ingredient, for example HSPDE10A or fragments thereof, antibodies of HSPDE10A, and agonists, antagonists or inhibitors of HSPDE10A, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the  
10   dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with  
15   little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the  
20   severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

25       Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells,  
30   conditions, locations, etc.

#### DIAGNOSTICS

In another embodiment, antibodies which specifically bind HSPDE10A may be used for the diagnosis of disorders characterized by expression of HSPDE10A, or in assays to monitor patients being treated with HSPDE10A or agonists, antagonists, or inhibitors of HSPDE10A. Antibodies  
35   useful for diagnostic purposes may be prepared in the same manner as described above for

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therapeutics. Diagnostic assays for HSPDE10A include methods which utilize the antibody and a label to detect HSPDE10A in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HSPDE10A, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HSPDE10A expression. Normal or standard values for HSPDE10A expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HSPDE10A under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HSPDE10A expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HSPDE10A may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HSPDE10A may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HSPDE10A, and to monitor regulation of HSPDE10A levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HSPDE10A or closely related molecules may be used to identify nucleic acid sequences which encode HSPDE10A. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HSPDE10A, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HSPDE10A encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:3-4 or from genomic sequences including promoters, enhancers, and introns of the HSPDE10A gene.

Means for producing specific hybridization probes for DNAs encoding HSPDE10A include the cloning of polynucleotide sequences encoding HSPDE10A or HSPDE10A derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available,

and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

5 Polynucleotide sequences encoding HSPDE10A may be used for the diagnosis of disorders associated with expression of HSPDE10A. Examples of such disorders include, but are not limited to, a cancer, such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; 10 and an immune disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, 15 psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma. The polynucleotide sequences encoding HSPDE10A may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HSPDE10A expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HSPDE10A may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide 30 sequences encoding HSPDE10A may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HSPDE10A in 35 the sample indicates the presence of the associated disorder. Such assays may also be used to

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evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HSPDE10A, a normal or standard profile for expression is established. This may be accomplished by  
5 combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HSPDE10A, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values  
10 obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from  
15 successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance  
20 of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HSPDE10A may involve the use of PCR. These oligomers may be chemically synthesized, generated  
25 enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HSPDE10A, or a fragment of a polynucleotide complementary to the polynucleotide encoding HSPDE10A, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

30 Methods which may also be used to quantify the expression of HSPDE10A include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is  
35 presented in various dilutions and a spectrophotometric or colorimetric response gives rapid

quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify  
 5 genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci.  
 10 USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HSPDE10A may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence.  
 15 The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

20 Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HSPDE10A on a physical chromosomal map and a specific disorder, or  
 25 a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps.  
 30 Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic  
 35 linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping

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to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

5 In another embodiment of the invention, HSPDE10A, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HSPDE10A and the agent being tested may be measured.

10 Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HSPDE10A, or fragments thereof, and washed. Bound HSPDE10A is then detected by methods well known in the art. Purified  
15 HSPDE10A can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HSPDE10A specifically compete with a test compound for binding  
20 HSPDE10A. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HSPDE10A.

In additional embodiments, the nucleotide sequences which encode HSPDE10A may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such  
25 properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

30 The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 09/226,741, are hereby expressly incorporated by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

35 The PROSNOT06 cDNA library was constructed from microscopically normal prostate tissue

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obtained from a 57-year-old Caucasian male. Pathology for the associated tumor indicated an adenocarcinoma (Gleason grade 3+3) in both the left and right periphery of the prostate. Perineural invasion was present, as was involvement of periprostatic tissue. Patient history included a benign neoplasm of the large bowel, appendectomy, and tonsillectomy with adenoidectomy. Family history  
5 included a malignant neoplasm of the prostate and type I diabetes.

The frozen tissue was homogenized and lysed in guanidinium isothiocyanate solution using a Polytron PT-3000 homogenizer (Brinkmann Instruments, Westbury NJ). The lysate was extracted once with an equal volume of acid phenol per Stratagene's RNA isolation protocol (Stratagene, San Diego CA). The RNA was extracted a second time with acid phenol, pH 4.7, precipitated using 0.3 M  
10 sodium acetate and 2.5 volumes of ethanol, resuspended in DEPC-treated water, and treated with DNase at 37°C for 25 minutes. mRNA was isolated using the OLIGOTEX kit (QIAGEN, Chatsworth CA) and used to construct the cDNA libraries. cDNAs were fractionated on a SEPHAROSE CL4B column (Amersham Pharmacia Biotech), and those cDNAs exceeding 400 bp were ligated into PSPORT1. The PSPORT1 plasmid was subsequently transformed into DH5 $\alpha$  competent cells (Life  
15 Technologies).

## II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge  
20 Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a  
25 high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

## 30 III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared

using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the

5 ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example IV.

10 The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 2 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 2 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third  
15 column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software  
20 (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA  
25 sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled  
30 into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS,  
35 DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such



as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:3-4. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Extension of HSPDE10A Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:3-4 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

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The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:3-4 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

#### V. Cloning of Full Length HSPDE10A

The complete cDNA insert from Incyte clone 826776 was isolated as a SalI/NotI restriction fragment, labeled with [ $\alpha$ -<sup>32</sup>P]dCTP, and used as a hybridization probe to screen  $\sim 1 \times 10^6$  plaque forming units from a human skeletal muscle 5'-STRETCH PLUS  $\lambda$ gt10 cDNA library (Clontech). Each cDNA insert was recovered as an EcoRI restriction fragment(s) and subcloned into PBLUESCRIPT KS+ (Stratagene). One  $\lambda$  clone (clone 1a.1) contained a 3.9 kb cDNA insert. Identification of a single, large open reading frame (Figures 1A, 1B, 1C, 1D, and 1E) allowed sequencing of both strands to produce the consensus nucleotide sequence, SEQ ID NO:3. HSPDE10A2, a C-terminal splice variant of HSPDE10A2 was also isolated by hybridization screening of the  $\lambda$  Clontech human skeletal muscle cDNA library. When the clone was isolated and fully sequenced, it revealed an insert with a 5' coding region similar to HSPDE10A1 and a 3' end similar to that of the original Incyte clone 826776 (Figures 2A, 2B, 2C, 2D, 2E, and 2F).

#### 35 VI. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

5 Membrane-based northern analysis was performed on RNA samples from a variety of human tissues using Multiple Tissue Northern blots (Clontech). For detecting human HSPDE10A, the ~1 kb cDNA insert of Incyte clone 826776 (SalI/NotI restriction fragment) was used. This comprises 108 bp 5' of the catalytic domain and 429 bp of the catalytic domain that is common to both HSPDE10A1 and HSPDE10A2. To examine HSPDE10A1 specifically, the ~1.7 kb EcoRI  
10 restriction fragment of  $\lambda$  clone 1a.1 which comprises 447 bp of the 3' portion of the catalytic domain and ~1.2 kb of the 3' untranslated region was used.

Each probe was labeled with [ $\alpha$ - $^{32}$ P]dCTP using a MEGAPRIME kit (Amersham, Buckinghamshire, UK) and reaction products (probe) were purified using CHROMASPIN-30 columns (Clontech). The Multiple Tissue Northern blots were pre-hybridized in EXPRESSHYB  
15 (Clontech) at 68°C for 1 hour and hybridised (~1 x 10<sup>6</sup> cpm probe/ml) at 68°C overnight. Blots were washed in 2 x SSPE, 0.05% (w/v) SDS at 50°C (4 x 15 min.) followed by 0.1 x SSPE, 0.1% (w/v) SDS at 50°C for 1 hour, and then exposed to film for 2-7 days. Blots were checked for equal loading of poly(A)<sup>+</sup> RNA in each lane using a human  $\beta$ -actin cDNA probe.

Northern analysis showed that HSPDE10A was expressed in skeletal muscle and prostate as  
20 a major transcript of ~7.5 kb; a ~3.0 kb mRNA was detected only in prostate; and a less prominent transcript of ~1.5 kb occurred in testes and skeletal muscle (Figures 5A and 5B). These data suggest that at least three PDE10A splice variants exist.

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This  
25 analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

100

30 The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

35 The results of northern analyses are reported as a percentage distribution of libraries in

which the transcript encoding HSPDE10A occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in the description of the invention.

#### VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:3-4 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

#### VIII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned

images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or  
 5 fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes  
 10 are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

#### IX. Complementary Polynucleotides

Sequences complementary to the HSPDE10A-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HSPDE10A. Although use of  
 15 oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HSPDE10A. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a  
 20 complementary oligonucleotide is designed to prevent ribosomal binding to the HSPDE10A-encoding transcript.

#### X. Subcloning and Expression of HSPDE10A

Two constructs encoding full length human HSPDE10A1 enzyme (plus and minus an N-terminal epitope tag) were generated for expression in insect cells using a baculovirus vector. Full  
 25 length human HSPDE10A1 was isolated by PCR from  $\lambda$  clone 1a.1 using a sense primer, 5'-CCAAATCCCGGTCCGAGATGTCCTCCCAAAGTGCAGTGCTGATGC-3' (SEQ ID NO:6), covering the initiation codon (underlined) and incorporating an RsrII restriction enzyme site, and an antisense primer, 5'-CGGGTACCTCGAGTTATTAGTTCCTGTCTTCCTTGGCTACC-3'; (SEQ ID NO:7), covering the termination codon (underlined) and incorporating a tandem stop codon and  
 30 unique XhoI restriction site. PCR was performed using the Expand High Fidelity PCR system (Boehringer Mannheim, West Sussex, UK) and the following cycle conditions: 94°C/1'45", 1 cycle; 94°C/15", 65°C/30", 72°C/1'45", 20 cycles, and 72°C/5', 1 cycle. The PCR product was digested with RsrII/XhoI and the resulting restriction fragment ligated into the RsrII/XhoI sites of both the baculovirus transfer vector PFASTBAC (Life Technologies) and PFASTBAC which had been  
 35 previously modified to include a 5' FLAG epitope tag (Kunz, D. et al. (1992) J. Biol. Chem.

267:9101-9106). The sequence of the insert for each construct was determined on both strands to confirm identity to the native HSPDE10A1 coding sequence, the encoded sequence being either native HSPDE10A1 or N-terminally FLAG-tagged HSPDE10A1.

Recombinant viral stocks were prepared using the Bac-to-Bac system (Life Technologies) according to the manufacturer's protocol, and Sf9 cells were cultured in Sf 900 II serum-free media (Life Technologies) at 27°C. For expression,  $3 \times 10^7$  cells in 30 ml were infected at a multiplicity of infection of 1. Cells were harvested 48 hours post-infection for assay. HSPDE10A for enzyme activity assays was prepared from transfected Sf9 cells harvested and disrupted by sonication. Cellular debris was removed by centrifugation at 12,000 x g for 15 min. followed by filtration (0.2 µm filter), and the clarified supernatant was dialyzed against 20 mM HEPES, pH 7.4, 1 mM EDTA, 150 mM NaCl at 4°C overnight. HSPDE10A1 was partially purified from the dialyzed supernatant by ion exchange chromatography using a 1 ml Mono Q HR (5/5) column (Pharmacia Biotech, Uppsala, Sweden). The column was eluted using a linear NaCl gradient up to 1M, and fractions containing high activity (>70% substrate turnover) were pooled and stored in aliquots at -70°C.

#### 15 **XI. PAGE and Western Analysis of HSPDE10A**

Transfected Sf9 cells were harvested by centrifugation (3,000 x g for 10 min.), resuspended in homogenization buffer (20 mM HEPES, pH 7.2, 1 mM EDTA, 20 mM sucrose, 150 mM NaCl and containing one protease inhibitor tablet (Boehringer) per 50 ml) at  $1 \times 10^7$  cells/ml, and disrupted by sonication. Cellular debris was removed by centrifugation at 12,000 x g for 15 min., and the supernatant was stored in aliquots at -70°C.

Human PDE10A infected and mock infected (control) cell lysates ( $\sim 6.4 \times 10^4$  cell equivalents for Coomassie staining, and  $\sim 640$  cell equivalents for western analysis) were separated by denaturing PAGE using the NuPAGE mini-gel system (Novex, San Diego CA) and either stained with Coomassie or transferred to a polyvinylidene difluoride membrane (Novex) for immunoblotting. Western analysis was performed by enhanced chemiluminescence (Amersham), according to the manufacturer's protocol, using an anti-FLAG antibody (Sigma, Dorset, UK) and a horse radish peroxidase conjugated anti-mouse IgG (Bio-Rad, Herts, UK) as a secondary antibody at 1:500 and 1:1,000 dilutions respectively.

#### **XII. Demonstration of HSPDE10A Activity**

PDE activity of HSPDE10A was measured using a Scintillation Proximity Assay (SPA)-based method employing a modification of the method of Hurwitz (Hurwitz, R.L. et al. (1984) J. Biol. Chem. 259:8612-8618). 50 µl of 20 mM Tris-HCl, pH 7.4 and 5 mM MgCl<sub>2</sub> containing the required concentration of cyclic nucleotide was added to 50 µl of diluted enzyme (or no enzyme for background control) in 20 mM Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>, and 2 mg/ml bovine serum albumin to

initiate the reaction. Both cAMP and cGMP were used as substrates (0.15-10  $\mu$ M final concentration) with a 3:1 ratio of unlabeled to [ $^3$ H]-labeled cAMP or cGMP (Amersham International). Reactions were performed in triplicate in Microfluor plates (Dynex Technologies, Chantilly VA) at 30°C for a period of time that would give less than 25% substrate turnover, to avoid non-linearity associated with product inhibition. The reaction was terminated by the addition of 50  $\mu$ l of PDE SPA beads (Yttrium Silicate, 20 mg/ml in water; Amersham International) along with a large excess (1 mM final concentration) of the respective unlabeled cyclic nucleotide (cGMP or cAMP). Plates were then sealed and shaken for 10 minutes to allow the beads to bind the nucleotide product. Finally, the SPA beads were allowed to settle for 30 minutes, and the plates read on a TopCount microtiter plate reader (Packard, Meriden CT).

To determine the  $K_m$  and  $V_{max}$  of the enzyme, the rate of hydrolysis of cAMP and cGMP was measured at a variety of substrate concentrations (i.e., 0.15-10  $\mu$ M) using a fixed amount of diluted enzyme over a time course of 5-60 minutes. Data points in the linear part of the reaction were then used to calculate  $K_m$  and  $V_{max}$  from a Michaelis-Menten plot using the "Fit Curve" Microsoft Excel extension program.

Inhibition studies were performed using the assay described above except that the appropriate inhibitor, dissolved and diluted as required in dimethylsulphoxide (DMSO), was added to the diluted enzyme to give the required final concentration (1-200  $\mu$ M). Reactions were initiated by the addition of substrate. cGMP was used as substrate at a final concentration of 0.17  $\mu$ M, a concentration equal to  $1/3 K_m$  so that  $IC_{50} \sim K_i$ . Sufficient enzyme was added to give ~25% substrate turnover during a 30 minute incubation at 30°C.

### XIII. Functional Assays

HSPDE10A function is assessed by expressing the sequences encoding HSPDE10A at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or

CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and  
 5 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

10 The influence of HSPDE10A on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HSPDE10A and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake  
 15 Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HSPDE10A and other genes of interest can be analyzed by northern analysis or microarray techniques.

#### XIV. Production of HSPDE10A Specific Antibodies

HSPDE10A substantially purified using polyacrylamide gel electrophoresis (PAGE; see,  
 20 e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HSPDE10A amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art.  
 25 Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase  
 30 immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HSPDE10A activity by, for example, binding the peptide or HSPDE10A to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.



**XV. Purification of Naturally Occurring HSPDE10A Using Specific Antibodies**

Naturally occurring or recombinant HSPDE10A is substantially purified by immunoaffinity chromatography using antibodies specific for HSPDE10A. An immunoaffinity column is constructed by covalently coupling anti-HSPDE10A antibody to an activated chromatographic resin, such as  
5 CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HSPDE10A are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HSPDE10A (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt  
10 antibody/HSPDE10A binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HSPDE10A is collected.

**XVI. Identification of Molecules Which Interact with HSPDE10A**

HSPDE10A, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate  
15 molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HSPDE10A, washed, and any wells with labeled HSPDE10A complex are assayed. Data obtained using different concentrations of HSPDE10A are used to calculate values for the number, affinity, and association of HSPDE10A with the candidate molecules.

20 Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are  
25 obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Inhibitor	Selective for PDE type (IC50)	IC50 for HSPDE10A1 ( $\mu$ M)
IBMX	non-selective (2-50 $\mu$ M)	40
Zaprinast	PDE5/6 (0.8/0.2 $\mu$ M)	8
Milrinone	PDE3 (1 $\mu$ M)	> 200
Rolipram	PDE4 (2.0 $\mu$ M)	160

Table 2

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater, fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLOCKS IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res. 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:38-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger, and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 2 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score $\geq$ GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <i>supra</i> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

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## SEQUENCE LISTING

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 PHILLIPS, Stephen C.  
 LANFEAR, Jerry  
 FAWCETT, Lindsay  
 BANDMAN, Olga  
 HARROW, Ian

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Asp	Ala	Tyr	Gln	Asp	Pro	Arg	Phe	Asp	Ala	Glu	Ala	Asp	Gln	Ile
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Ser	Asn	His	Gln	Ile	Ile	Gly	Val	Ala	Gln	Val	Leu	Asn	Arg	Leu
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Asp	Gly	Lys	Pro	Phe	Asp	Asp	Ala	Asp	Gln	Arg	Leu	Phe	Glu	Ala
			95						100					105
Phe	Val	Ile	Phe	Cys	Gly	Leu	Gly	Ile	Asn	Asn	Thr	Ile	Met	Tyr
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Asp	Gln	Val	Lys	Lys	Ser	Trp	Ala	Lys	Gln	Ser	Val	Ala	Leu	Asp
			125						130					135
Val	Leu	Ser	Tyr	His	Ala	Thr	Cys	Ser	Lys	Ala	Glu	Val	Asp	Lys
			140						145					150
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185	190	195
Phe Lys Ile Asp Tyr Glu Thr Leu Cys	Arg Trp Leu Leu Thr Val	
200	205	210
Arg Lys Asn Tyr Arg Met Val Leu Tyr	His Asn Trp Arg His Ala	
215	220	225
Phe Asn Val Cys Gln Leu Met Phe Ala	Met Leu Thr Thr Ala Gly	
230	235	240
Phe Gln Asp Ile Leu Thr Glu Val Glu	Ile Leu Ala Val Ile Val	
245	250	255
Gly Cys Leu Cys His Asp Leu Asp His	Arg Gly Thr Asn Asn Ala	
260	265	270
Phe Gln Ala Lys Ser Gly Ser Ala Leu	Ala Gln Leu Tyr Gly Thr	
275	280	285
Ser Ala Thr Leu Glu His His His Phe	Asn His Ala Val Met Ile	
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Leu Gln Ser Glu Gly His Asn Ile Phe	Ala Asn Leu Ser Ser Lys	
305	310	315
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Asp Ile Phe Arg Ser Met Leu Met Thr	Ala Cys Asp Leu Gly Ala	
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Lys Leu Thr Pro Ser Ala Ile Phe Asp	Arg Asn Arg Lys Asp Glu	
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Leu Pro Arg Leu Gln Leu Glu Trp Ile	Asp Ser Ile Cys Met Pro	
425	430	435
Leu Tyr Gln Ala Leu Val Lys Val Asn	Val Lys Leu Lys Pro Met	
440	445	450
Leu Asp Ser Val Ala Thr Asn Arg Ser	Lys Trp Glu Glu Leu His	
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Gln Lys Arg Leu Leu Ala Ser Thr Ala	Ser Ser Ser Ser Pro Ala	
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&lt;211&gt; 367

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&lt;220&gt;

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Ser Gly Phe His Ile Arg Ser Val Leu Cys Val Pro Ile Trp Asn
65          70          75
Ser Asn His Gln Ile Ile Gly Val Ala Gln Val Leu Asn Arg Leu
80          85          90
Asp Gly Lys Pro Phe Asp Asp Ala Asp Gln Arg Leu Phe Glu Ala
95          100         105
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170         175         180
Thr Ala Ala Leu Arg Met Phe Met Glu Leu Gly Met Val Gln Lys
185         190         195
Phe Lys Ile Asp Tyr Glu Thr Leu Cys Arg Trp Leu Leu Thr Val
200         205         210
Arg Lys Asn Tyr Arg Met Val Leu Tyr His Asn Trp Arg His Ala
215         220         225
Phe Asn Val Cys Gln Leu Met Phe Ala Met Leu Thr Thr Ala Gly
230         235         240
Phe Gln Asp Ile Leu Thr Glu Val Glu Ile Leu Ala Val Ile Val
245         250         255
Gly Cys Leu Cys His Asp Leu Asp His Arg Gly Thr Asn Asn Ala
260         265         270
Phe Gln Ala Lys Ser Gly Ser Ala Leu Ala Gln Leu Tyr Gly Thr
275         280         285
Ser Ala Thr Leu Glu His His His Phe Asn His Ala Val Met Ile
290         295         300
Leu Gln Ser Glu Gly His Asn Ile Phe Ala Asn Leu Ser Ser Lys
305         310         315
Glu Tyr Ser Asp Leu Met Gln Leu Leu Lys Gln Ser Ile Leu Ala
320         325         330
Thr Asp Leu Thr Leu Tyr Phe Glu Glu Lys Val Arg Asn Thr Ser
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WO 00/40733

PCT/US00/00371

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Ser Thr Leu Glu Glu Val Ser Asn Asn Cys Ile Arg Leu Glu Trp		
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Ile Lys Asn His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile		
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Gln Val Leu Leu Asp Leu Ala Ser Leu Ile Phe Glu Glu Gln Gln		
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Ser Leu Glu Val Ile Leu Lys Lys Ile Ala Ala Thr Ile Ile Ser		
350	355	360
Phe Met Gln Val Gln Lys Cys Thr Ile Phe Ile Val Asp Glu Asp		
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Cys Ser Asp Ser Phe Ser Ser Val Phe His Met Glu Cys Glu Glu		
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Leu Glu Lys Ser Ser Asp Thr Leu Thr Arg Glu His Asp Ala Asn		
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Lys Ile Asn Tyr Met Tyr Ala Gln Tyr Val Lys Asn Thr Met Glu		
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Pro Leu Asn Ile Pro Asp Val Ser Lys Asp Lys Arg Phe Pro Trp		
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Thr Thr Glu Asn Thr Gly Asn Val Asn Gln Gln Cys Ile Arg Ser		
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Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val Ile		
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Gly Val Cys Gln Leu Val Asn Lys Met Glu Glu Asn Thr Gly Lys		
470	475	480
Val Lys Pro Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu Ala Phe		
485	490	495
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770	775	780
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785	790	795
Glu Leu Asn Ile Glu Pro Thr Asp Leu Met Asn Arg Glu Lys Lys		
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Pro Leu Leu Asp Gly Cys Arg Lys Asn Arg Gln Lys Trp Gln Ala		
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&lt;211&gt; 43

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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PCT/US00/00371

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID: sense primer

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43

&lt;210&gt; 7

&lt;211&gt; 41

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID: antisense primer

&lt;400&gt; 7

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41

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What is claimed is:

1. An isolated polypeptide comprising:
  - a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-2,
  - 5 b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2,
  - c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or
  - d) an immunogenic fragment of an amino acid sequence selected from the group consisting  
10 of SEQ ID NO:1-2.
2. An isolated polypeptide of claim 1, having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.
- 15 3. An isolated polynucleotide encoding a polypeptide of claim 1.
4. An isolated polynucleotide of claim 3, having a sequence selected from the group consisting of SEQ ID NO:3-4.
- 20 5. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.
6. A cell transformed with a recombinant polynucleotide of claim 5.
- 25 7. A transgenic organism comprising a polynucleotide of claim 5.
8. A method for producing a polypeptide of claim 1, the method comprising:
  - a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide  
30 comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and
  - b) recovering the polypeptide so expressed.
9. An isolated antibody which specifically binds to a polypeptide of claim 1.

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10. An isolated polynucleotide comprising:
- a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4,
  - b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4,
  - 5 c) a polynucleotide sequence complementary to a), or
  - d) a polynucleotide sequence complementary to b).
11. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 10.
12. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 10, the method comprising:
- a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe
  - 15 specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and
  - b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.
13. A method of claim 12, wherein the probe comprises at least 30 contiguous nucleotides.
14. A method of claim 12, wherein the probe comprises at least 60 contiguous nucleotides.
15. A pharmaceutical composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.
16. A method of treating a disease or condition associated with decreased expression of functional HSPDE10A, comprising administering to a patient in need of such treatment the pharmaceutical composition of claim 15.
17. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:
- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
  - b) detecting agonist activity in the sample.

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18. A pharmaceutical composition comprising an agonist compound identified by a method of claim 17 and a pharmaceutically acceptable excipient.

5 19. A method of treating a disease or condition associated with decreased expression of functional HSPDE10A, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 18.

20. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

10 a) exposing a sample comprising a polypeptide of claim 1 to a compound, and  
b) detecting antagonist activity in the sample.

21. A pharmaceutical composition comprising an antagonist compound identified by a method of claim 20 and a pharmaceutically acceptable excipient.

15

22. A method for treating a disease or condition associated with overexpression of functional HSPDE10A, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 21.

20 23. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 4, the method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, and  
b) detecting altered expression of the target polynucleotide.

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9 18 27 36 45 54  
 5' TGG AAA GAT GTT ACT TCA TCT CCC AGG TTT GCT CAC TGC AAA TAC AAT CCT GAG

63 72 81 90 99 108  
 AAC TGA ACT AGG GCC TTA AAG TCC TGA CAT GCA TGG CTT GGT TTT GTG GAT TGC

117 126 135 144 153 162  
 CTC TCT CAA CAG GTG GTG AAA TTT ACC AAA TCC TTT GAA TTG ATG TCC CCA AAG

171 180 189 198 207 216  
 TGC AGT GCT GAT GCT GAG AAC AGT TTC AAA GAA GAG AAA TCA TCA TAC

225 234 243 252 261 270  
 TCC GAC TGG CTA ATA AAT AAC AGC ATT GCT GAG CTG GTT GCT TCA ACA GGC CTT

279 288 297 306 315 324  
 CCA GTG AAC ATC AGT GAT GCC TAC CAG GAT CCG CGC TTT GAT GCA GAG GCA GAC

333 342 351 360 369 378  
 CAG ATA TCT GGT TTT CAC ATA AGA TCT GTT CTT TGT GTC CCT ATT TGG AAT AGC

Q I S G F H I R S V L C V P I W N S

FIGUREIA



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387	396	405	414	423	432
AAC CAC CAA ATA ATT GGA GTG GCT CAA GTG TTA AAC AGA CTT GAT GGG AAA CCT					
N H Q I I I G V A Q Q V L N R L D G K P					
441	450	459	468	477	486
TTT GAT GAT GCA GAT CAA CGA CTT TTT GAG GCT TTT GTC ATC TTT TGT GGA CTT					
F D D A D Q Q R L F E A F V I F C G L					
495	504	513	522	531	540
GGC ATC AAC AAC ACA ATT ATG ATG CAA GTG AAG AAG TCC TGG GCC AAG CAG					
G I N N T I M Y D Q V K K S W A K Q					
549	558	567	576	585	594
TCT GTG GCT CTT GAT GTG CTA TCA TAC CAT GCA ACA TGT TCA AAA GCT GAA GTT					
S V A L D V V L S Y H A T C S K A E V					
603	612	621	630	639	648
GAC AAG TTT AAG GCA GCC AAC ATC CCT CTG GTG TCA GAA CTT GCC ATC GAT GAC					
D K F K A A N I P L V S E L A I D D					
657	666	675	684	693	702
ATT CAT TTT GAT GAC TTT TCT CTC GAC GTT GAT GCC ATG ATC ACA GCT GCT CTC					
I H F D D F S L D D V D A M I T A A L					
711	720	729	738	747	756
CGG ATG TTC ATG GAG CTG GGG ATG GTA CAG AAA TTT AAA ATT GAC TAT GAG ACA					
R M F M E L G M V Q K F K I D Y E T					

FIGURE 1B

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765	774	783	792	801	810
CTG TGT AGG TGG CTT TTG ACA GTG AGG AAA AAC TAT CGG ATG GTT CTA TAC CAC					
L C R W L L T V R R K N Y R M V L Y H					
819	828	837	846	855	864
AAC TGG AGA CAT GCC TTC AAC GTG TGT CAG CTG ATG TTC GCG ATG TTA ACC ACT					
N W R H A F N V C Q L M F A M L T T					
873	882	891	900	909	918
GCT GGG TTT CAA GAC ATT CTG ACC GAG GTG GAA ATT TTA GCG GTG ATT GTG GGA					
A G F Q D I L T E V E I L A V I V G					
927	936	945	954	963	972
TGC CTG TGT CAT GAC CTC GAC CAC AGG GGA ACC AAC AAT GCC TTC CAA GCT AAG					
C L C H D L D H R G T N N A F Q A K					
981	990	999	1008	1017	1026
AGT GGC TCT GCC CTG GCC CAA CTC TAT GGA ACC TCT GCT ACC TTG GAG CAT CAC					
S G S A L A Q L Y G T S A T L E H H					
1035	1044	1053	1062	1071	1080
CAT TTC AAC CAC GCC GTG ATG ATC CTT CAA AGT GAG GGT CAC AAT ATC TTT GCT					
H F N H A V M I L Q S E G H N I F A					
1089	1098	1107	1116	1125	1134
AAC CTG TCC TCC AAG GAA TAT AGT GAC CTT ATG CAG CTT TTG AAG CAG TCA ATA					
N L S S K E Y S D L M Q L L K Q S I					

FIGURE 1C

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1143	1152	1161	1170	1179	1188
TTG GCA ACA GAC CTC ACG CTG TAC TTT GAG AGG AGA ACT GAA TTC TTT GAA CTT					
L A T D L T L Y F E R R T E F E L					
1197	1206	1215	1224	1233	1242
GTC AGT AAA GGA GAA TAC GAT TGG AAC ATC AAA AAC CAT CGT GAT ATA TTT CGA					
V S K G E Y D W N I K N H R D I F R					
1251	1260	1269	1278	1287	1296
TCA ATG TTA ATG ACA GCC TGT GAC CTT GGA GCC GTG ACC AAA CCG TGG GAG ATC					
S M L M T A C D L G A V T K P W E I					
1305	1314	1323	1332	1341	1350
TCC AGA CAG GTG GCA GAA CTT GTA ACC AGT GAG TTC TTC GAA CAA GGA GAT CGG					
S R Q V A E L V T S E F F E Q G D R					
1359	1368	1377	1386	1395	1404
GAG AGA TTA GAG CTC AAA CTC ACT CCT TCA GCA ATT TTT GAT CGG AAC CGG AAG					
E R L E L K L T P S A I F D R N R K					
1413	1422	1431	1440	1449	1458
GAT GAA CTG CCT CGG TTG CAA CTG GAG TGG ATT GAT AGC ATC TGC ATG CCT TTG					
D E L P R L Q L E W I D S I C M P L					
1467	1476	1485	1494	1503	1512
TAT CAG GCA CTG GTG AAG GTC AAC GTG AAA CTG AAG CCG ATG CTA GAT TCA GTA					
Y Q A L V K V N V K L K K P M L D S V					

FIGURE1D

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1521	1530	1539	1548	1557	1566
GCT ACA AAC AGA AGT AAG TGG GAA GAG CTA CAC CAA AAA CGA CTG CTG GCC TCA					
A T N R S K W E E L H Q K R L L A S					
1575	1584	1593	1602	1611	1620
ACT GCC TCA TCC TCC CCT GCC AGT GTT ATG GTA GCC AAG GAA GAC AGG AAC					
T A S S S S P A S V M V A K E D R N					
1629	1638	1647	1656	1665	1674
TAA ACC TCC AGG TCA GCT GCA GCT GCA AAA TGA CTA CAG CCT GAA GGG CCA TTT					
1683	1692	1701	1710	1719	1728
TCA GTC CAG CAA TGT CAT CCT TTT GTT CTT TTA GCT CAG AAA GAC CTA ACA TCT					
1737	1746	1755	1764	1773	1782
CAA GGA TGC ACT GGG AAC CAT GCC TGG GCT TTC ACC TTG AAG CAT GGT CAG CAG					

CA 3'

FIGURE 1E

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5' TCG ACG TGG AAA GAT GTT ACT TCA TCT CCC AGG TTT GCT CAC TGC AAA TAC AAT  
 9 18 27 36 45 54  
 CCT GAG AAC TGA ACT AGG GCC TTA AAG TCC TGA CAT GCA TGG CTT GGT TTT GTG  
 63 72 81 90 99 108  
 GAT TGC CTC TCT CAA CAG GTG GTG AAA TTT ACC AAA TCC TTT GAA TTG ATG TCC  
 117 126 135 144 153 162  
 CCA AAG TGC AGT GCT GAT GCT GAG AAC AGT TTC AAA GAA AGC ATG GAG AAA TCA  
 171 180 189 198 207 216  
 TCA TAC TCC GAC TGG CTA ATA AAT AAC AGC AGT ATT GCT GAG CTG GTT GCT TCA ACA  
 225 234 243 252 261 270  
 GGC CTT CCA GTG AAC ATC AGT GAT GCC TAC CAG GAT CCG CGC TTT GAT GCA GAG  
 279 288 297 306 315 324  
 GCA GAC CAG ATA TCT GGT TTT CAC ATA AGA TCT GTT CTT TGT GTC CCT ATT TGG  
 333 342 351 360 369 378  
 A D Q I S G F H I R S V L C V P I W

FIGURE2A

387	396	405	414	423	432
AAT AGC AAC CAC CAA ATA ATT GGA GTG CAA GTG TTA AAC AGA CTT GAT GGG					
N S N H Q I I G V A Q V L N R L D G					
441	450	459	468	477	486
AAA CCT TTT GAT GAT GCA GAT CAA CGA CTT TTT GAG GCT TTT GTC ATC TTT TGT					
K P F D D A D Q Q R L F E A F I F C					
495	504	513	522	531	540
GGA CTT GGC ATC AAC AAC ACA ATT ATG TAT GAT CAA GTG AAG AAC TCC TGG GCC					
G L G I N N T I M Y D Q V K K S W A					
549	558	567	576	585	594
AAG CAG TCT GTG GCT CTT GAT GTG CTA TCA TAC CAT GCA ACA TGT TCA AAA GCT					
K Q S V A L D V L S Y H A T C S K A					
603	612	621	630	639	648
GAA GTT GAC AAG TTT AAG GCA GCC AAC ATC CCT CTG GTG TCA GAA CTT GCC ATC					
E V D K K F K A A N I P L V S E L A I					
657	666	675	684	693	702
GAT GAC ATT CAT TTT GAT GAC TTT TCT CTC GAC GTT GAT GCC ATG ATC ACA GCT					
D D I H F D D F S L D V D A M I T A					
711	720	729	738	747	756
GCT CTC CGG ATG TTC ATG GAG CTG GCG ATG GTA CAG AAA TTT AAA ATT GAC TAT					
A L R M F M E L G M V Q K F K I D Y					

FIGURE2B

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765	774	783	792	801	810
GAG ACA CTG TGT AGG TGG CTT TTG ACA GTG AGG AAA AAC TAT CGG ATG GTT CTA					
E T L C R W L L T V R K N Y R M V L					
819	828	837	846	855	864
TAC CAC AAC TGG AGA CAT GCC TTC AAC GTG TGT CAG CTG ATG TTC GCG ATG TTA					
Y H N W R H A F N V C Q L M F A M L					
873	882	891	900	909	918
ACC ACT GCT GGG TTT CAA GAC ATT CTG ACC GAG GTG GAA ATT TTA GCG GTG ATT					
T T A G F Q D I L T E V E I L A V I					
927	936	945	954	963	972
GTG GGA TGC CTG TGT CAT GAC CTC GAC CAC AGG GGA ACC AAC AAT GCC TTC CAA					
V G C L C H D L D H R G T N N A F Q					
981	990	999	1008	1017	1026
GCT AAG AGT GGC TCT GCC CTG GCC CAA CTC TAT GGA ACC TCT GCT ACC TTG GAG					
A K S G S A L A Q L Y G T S A T L E					
1035	1044	1053	1062	1071	1080
CAT CAC CAT TTC AAC CAC GCC GTG ATG ATC CTT CAA AGT GAG GGT CAC AAT ATC					
H H H F N H A V M I L Q S E G H N I					
1089	1098	1107	1116	1125	1134
TTT GCT AAC CTG TCC TCC AAG GAA TAT AGT GAC CTT ATG CAG CTT TTG AAG CAG					
F A N L S S K E Y S D L M Q L L K Q					

FIGURE2C

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1143	1152	1161	1170	1179	1188
TCA ATA TTG GCA ACA GAC CTC ACG CTG TAC TTT GAG GAG AAG GTC AGA AAT ACA					
S I L A T D L T L Y F E E K V R N T					
1197	1206	1215	1224	1233	1242
TCA CCT GGA GCT GTG AAC CAC CTA CCT GGC ACA AGC AAT CTG CAG CTC TTC TTT					
S P G A V N H L P G T S N L Q L F F					
1251	1260	1269	1278	1287	1296
GGA GCA CCC CCT TAT TGA TGA TGG AAA GAA CCC TGT CTG TGT CTG CCT TGA TAC					
G A P P Y					
1305	1314	1323	1332	1341	1350
TTG GTA TTG CCT TGG TAC AGC AGC CTG TGA TGC TGT TAC ATA GCA TGA GGG CTG					
1359	1368	1377	1386	1395	1404
CTG GCC CCA CTG TCC ATA CAC TTA CAA CAT GAA AAG CTA TCT GGC CCA AAG GTT					
1413	1422	1431	1440	1449	1458
TAT GCT ACA CAT AGT TTA CAA AGA TTA TCT CAG AGG GCA GAA CCG GGA GGC TGG					
1467	1476	1485	1494	1503	1512
GGA CTT ATA ATC TAC CCA GAA GGA AAA GTT CTT CCT TAT AGA AGA TTT CAA TTA					

FIGURE 2D



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1521	1530	1539	1548	1557	1566
ACA CAC ATG GAA AGG TGG AAA TGG AAA AAT CAT CAG CTG GCA AAT ACC ACG GTA					
1575	1584	1593	1602	1611	1620
GTA ATT TTT ATT GGC AAC AAT AAA TCT TTC TGT AAC TGC CCT GGG ACC TTG AAC					
1629	1638	1647	1656	1665	1674
AAG TCA CTT CAC CTT CCT TCA CCT TGA GTT TCC TCA CCT ATA AAA TGA GAG AAT					
1683	1692	1701	1710	1719	1728
TAA TAG GAG ATT TTT CTC AAA AGT TCC ATA CAG CCC TAC CAG TCT ATA ACT ATA					
1737	1746	1755	1764	1773	1782
ATG AAA ATT CAA ACA TAG AAA AGA AGT CAT TCT ATG ACC CAG CAA TTT TAC ATA					
1791	1800	1809	1818	1827	1836
TAC ATG TAC ATA TTC ATA TAC ACA GAG AGA GAG AAC TCA CAC AAA TTC ACA AGG					

FIGURE 2E

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1845	1854	1863	1872	1881	1890
AAA CAT GTA CAA GGT GGT TCA TAG CTG CAT TGT ATG TAA TAG CAA GAA ATA TTA					
1899	1908	1917	1926	1935	1944
GAA AAA TAT AAA TTT TCA TCT TCC AGG AAA TGG GTA AAT AGA CAG TGG TAT AAT					
1953	1962	1971	1980		
AAT AGA TGG AAA TAG CAT ACA TCA GTA TGA AGG AAT GG 3'					

FIGURE 2F

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1	M S	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
1	M S	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
1	M E R A G P S F G Q Q R Q Q Q P Q Q K Q Q Q R D Q D S V	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1
3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
31	E A W L D D H W D F T F S Y F V R K A T R E M V N A W F A E	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1
3	- - - - -	P K C S	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
3	- - - - -	P K C S	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
61	R V H T I P V C K E G I R G H T E S C S C P L Q Q S P R A D	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1
7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	A D A	HSPDE10A1
7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	A D A	HSPDE10A2
91	N S V P G T P T R K I S A S E F D R P L R P I V V K D S E G	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1
10	E N S F K E S M E K S S	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
10	E N S F K E S M E K S S	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
121	T V S F L S D S E K K E Q M P L T P P R F D H D E G D Q C S	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1
22	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
22	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
151	R L L E L V K D I S S H L D V T A L C H K I F L H I H G L I	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HPDE5A1

**FIGURE 3A**

22	- - - Y S D W L I - - - - - - - - - - - - - - - HSPDE10A1
22	- - - Y S D W L I - - - - - - - - - - - - - - - HSPDE10A2
181	S A D R Y S L F L V C E D S S N D K F L I S R L F D V A E G HPDE5A1
28	- - - - - - - - - - - N N S I A E L V A S T G L P V HSPDE10A1
28	- - - - - - - - - - - N N S I A E L V A S T G L P V HSPDE10A2
211	S T L E E V S N N C I R L E W N K G I V G H V A A L G E P L HPDE5A1
43	N I S D A Y Q D P R F D A E A D Q I S G F H I R S V L C V P HSPDE10A1
43	N I S D A Y Q D P R F D A E A D Q I S G F H I R S V L C V P HSPDE10A2
241	N I K D A Y E D P R F N A E V D Q I T G Y K T Q S I L C M P HPDE5A1
73	I - HSPDE10A1
73	I - HSPDE10A2
271	I K N H R E E V G V A Q A I N K K S G N G G T F T E K D E HPDE5A1
74	- HSPDE10A1
74	- HSPDE10A2
301	K D F A A Y L A F C G I V L H N A Q L Y E T S L L E N K R N HPDE5A1
74	- HSPDE10A1
74	- HSPDE10A2
331	Q V L L D L A S L I F F E E Q Q S L E V I L K K I A A T I I S HPDE5A1

**FIGURE 3B**

74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
361	F M Q V Q K C T I F I V D E D C S D S F S S V F H M E C E E					HPDE5A1
74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
391	L E K S S D T L T R E H D A N K I N Y M Y A Q Y V K N T M E					HPDE5A1
74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
74	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
421	P L N I P D V S K D K R F P W T T E N T G N V N Q Q C I R S					HPDE5A1
79	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
79	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
451	L L C T P I K N G K K N K V I G V C Q L V N K M E E N T G K					HPDE5A1
93	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A1
93	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	HSPDE10A2
481	V K P F N R N D E Q F L E A F V I F C G L G I Q N T Q M Y E					HPDE5A1
122	Q V K K S W A K Q S V A L D V L S Y H A T C S K A E					HSPDE10A1
122	Q V K K S W A K Q S V A L D V L S Y H A T C S K A E					HSPDE10A2
511	A V E R A M A K Q M V T L E V L S Y H A S A A E E E T R E L					HPDE5A1

**FIGURE 3C**

149	D	K	F	K	A	A	N	I	P	L	V	S	E	L	A	I	D	D	I	H	F	D	D	F	S	L	D	V	D	A	HSPDE10A1	
149	D	K	F	K	A	A	N	I	P	L	V	S	E	L	A	I	D	D	I	H	F	D	D	F	S	L	D	V	D	A	HSPDE10A2	
541	Q	S	L	A	A	V	P	S	A	Q	T	L	K	I	T	D	F	S	F	S	D	F	E	L	S	D	L	E		HPDE5A1		
179	M	I	T	A	A	L	R	M	F	M	E	L	G	M	V	Q	K	F	K	I	D	Y	E	T	L	C	R	W	L	L	HSPDE10A1	
179	M	I	T	A	A	L	R	M	F	M	E	L	G	M	V	Q	K	F	K	I	D	Y	E	T	L	C	R	W	L	L	HSPDE10A2	
571	T	A	L	C	T	I	R	M	F	T	D	L	N	L	V	Q	N	F	Q	M	K	H	E	V	L	C	R	W	I	L	HPDE5A1	
209	T	V	R	K	N	Y	R	M	-	V	L	Y	H	N	W	R	R	H	A	F	N	V	C	Q	L	M	F	A	M	L	T	HSPDE10A1
209	T	V	R	K	N	Y	R	M	-	V	L	Y	H	N	W	R	R	H	A	F	N	V	C	Q	L	M	F	A	M	L	T	HSPDE10A2
601	S	V	K	K	N	Y	R	K	N	V	A	Y	H	N	W	R	R	H	A	F	N	T	A	Q	C	M	F	A	A	L	K	HPDE5A1
238	T	A	G	F	Q	D	I	L	T	E	V	E	I	L	A	V	I	V	G	C	L	C	H	D	L	D	H	R	G	T	HSPDE10A1	
238	T	A	G	F	Q	D	I	L	T	E	V	E	I	L	A	V	I	V	G	C	L	C	H	D	L	D	H	R	G	T	HSPDE10A2	
631	A	G	K	I	Q	N	K	L	T	D	L	E	I	L	A	L	I	A	A	L	S	H	D	L	D	H	R	G	V	HPDE5A1		
268	N	N	A	F	Q	A	K	S	G	S	A	L	A	Q	L	Y	G	T	S	A	T	L	E	H	H	H	F	N	H	A	HSPDE10A1	
268	N	N	A	F	Q	A	K	S	G	S	A	L	A	Q	L	Y	G	T	S	A	T	L	E	H	H	H	F	N	H	A	HSPDE10A2	
661	N	N	S	I	Q	R	S	E	H	P	L	A	Q	L	Y	C	H	S	-	I	M	E	H	H	H	F	D	Q	C	HPDE5A1		
298	V	M	I	L	Q	S	E	G	H	N	I	F	A	N	L	S	S	K	E	Y	S	D	L	M	Q	L	L	K	Q	S	HSPDE10A1	
298	V	M	I	L	Q	S	E	G	H	N	I	F	A	N	L	S	S	K	E	Y	S	D	L	M	Q	L	L	K	Q	S	HSPDE10A2	
690	L	M	I	L	N	S	P	G	N	Q	I	L	S	G	L	S	I	E	E	Y	K	T	T	L	K	I	I	K	Q	A	HPDE5A1	

FIGURE 3D

328	I	L	A	T	D	L	T	L	Y	F	E	R	R	T	E	F	F	E	L	V	S	K	G	E	Y	D	W	N	I	K	HSPDE10A1
328	I	L	A	T	D	L	T	L	Y	F	E	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R	HSPDE10A2	
720	I	L	A	T	D	L	A	L	Y	I	K	R	R	G	E	F	F	E	L	I	R	K	N	Q	F	N	L	E	D	P	HPDE5A1
358	N	H	R	D	I	F	R	S	M	L	T	A	C	D	L	G	A	V	T	K	P	W	E	I	S	R	Q	V	A	HSPDE10A1	
343	N	T	S	P	G	A	V	N	H	L	P	G	T	S	N	L	-	-	-	-	-	-	-	-	-	-	-	-	-	HSPDE10A2	
750	H	Q	K	E	L	F	L	A	M	L	M	T	A	C	D	L	S	A	I	T	K	P	W	P	I	Q	Q	R	I	A	HPDE5A1
388	E	L	V	T	S	E	F	F	E	Q	G	D	R	E	R	L	E	L	K	L	T	P	S	A	I	F	D	R	N	R	HSPDE10A1
359	Q	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	HSPDE10A2	
780	E	L	V	A	T	E	F	F	D	Q	G	D	R	E	R	K	E	L	N	I	E	P	T	D	L	M	N	R	E	K	HPDE5A1
418	K	D	E	L	P	R	L	Q	L	E	W	I	D	S	I	C	M	P	L	Y	Q	A	L	V	K	V	N	V	K	L	HSPDE10A1
365	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	Y	HSPDE10A2								
810	K	N	K	I	P	S	M	Q	V	G	F	I	D	A	I	C	L	Q	L	Y	E	A	L	T	H	V	S	E	D	C	HPDE5A1
448	K	P	M	L	D	S	V	A	T	N	R	S	K	W	E	E	L	-	-	H	Q	K	R	L	L	A	S	T	A	S	HSPDE10A1
367	F	P	L	L	D	G	C	R	K	N	R	Q	K	W	Q	A	L	A	E	Q	Q	E	K	M	L	I	N	G	E	S	HSPDE10A2
840	F	P	L	L	D	G	C	R	K	N	R	Q	K	W	Q	A	L	A	E	Q	Q	E	K	M	L	I	N	G	E	S	HPDE5A1
476	S	S	S	P	A	S	V	M	V	A	K	E	D	R	N	HSPDE10A1															
367	S	S	S	P	A	S	V	M	V	A	K	E	D	R	N	HSPDE10A2															
870	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	HPDE5A1	

FIGURE 3E

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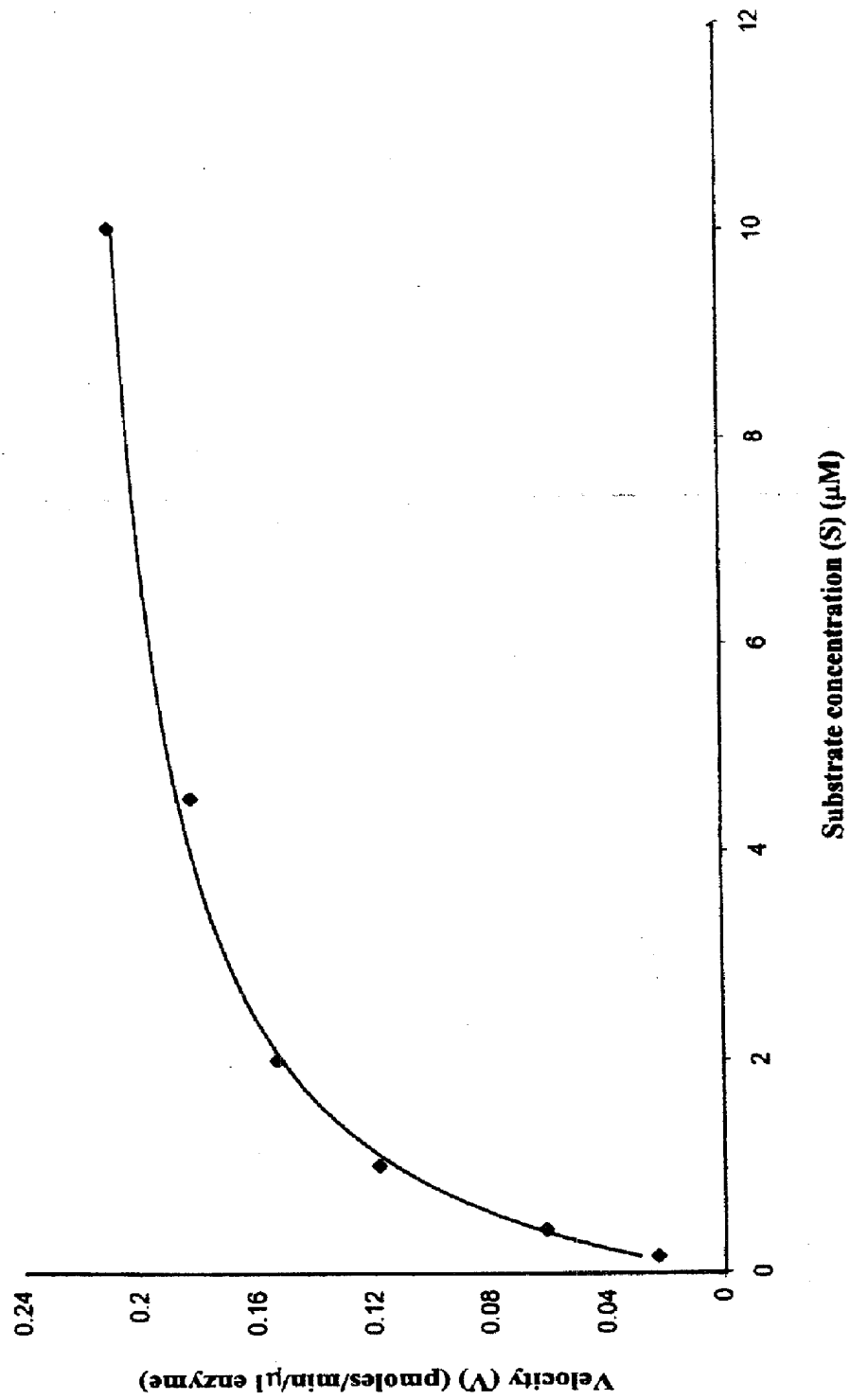


FIGURE 4A



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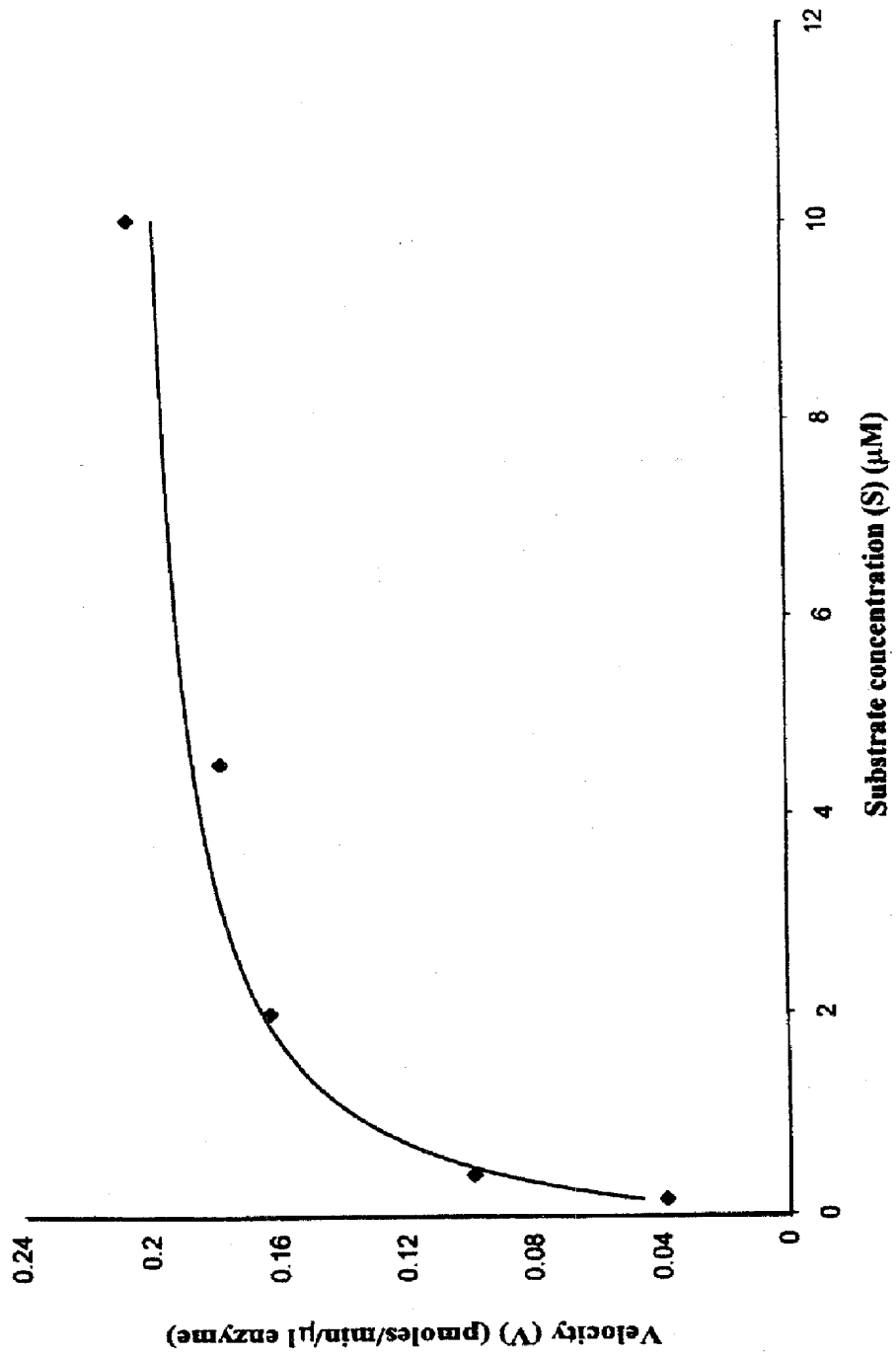


FIGURE 4B

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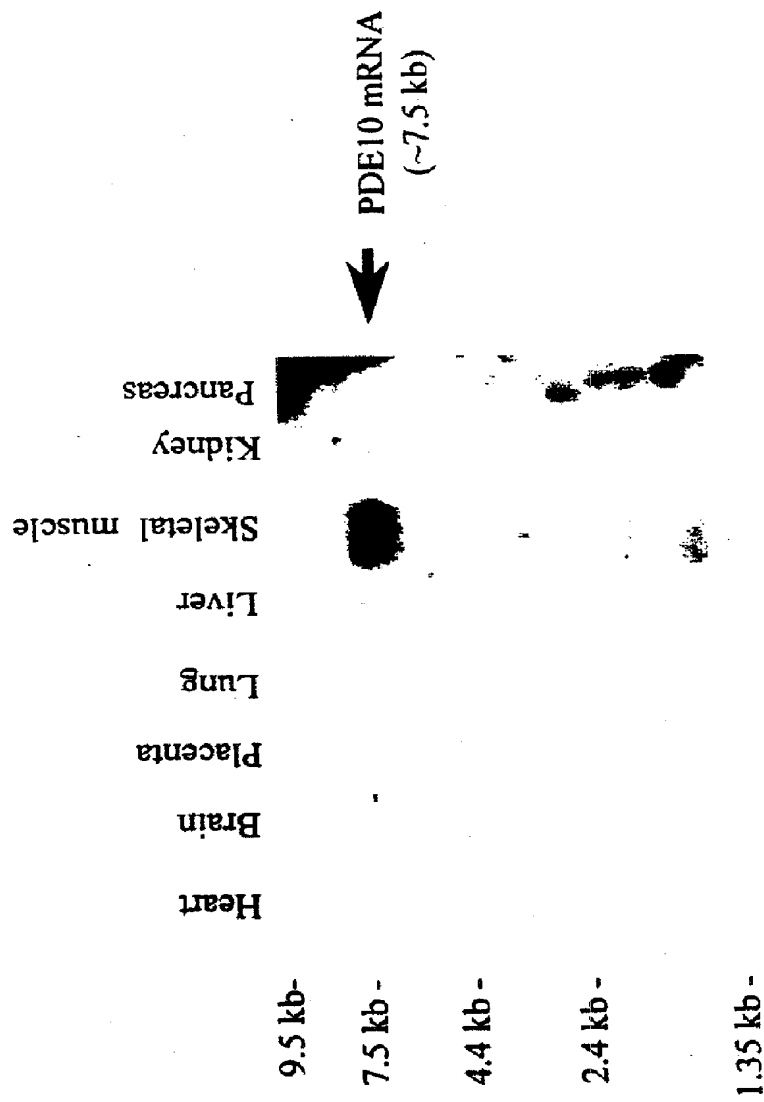


FIGURE 5A

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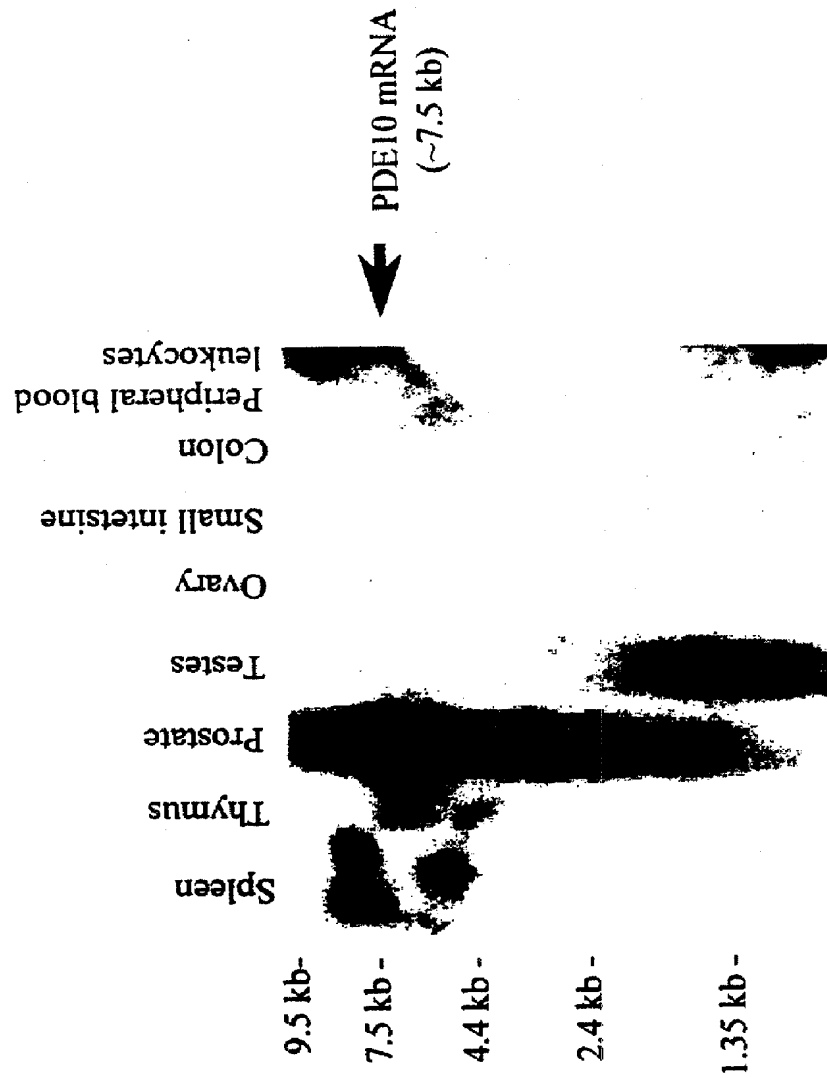


FIGURE 5B

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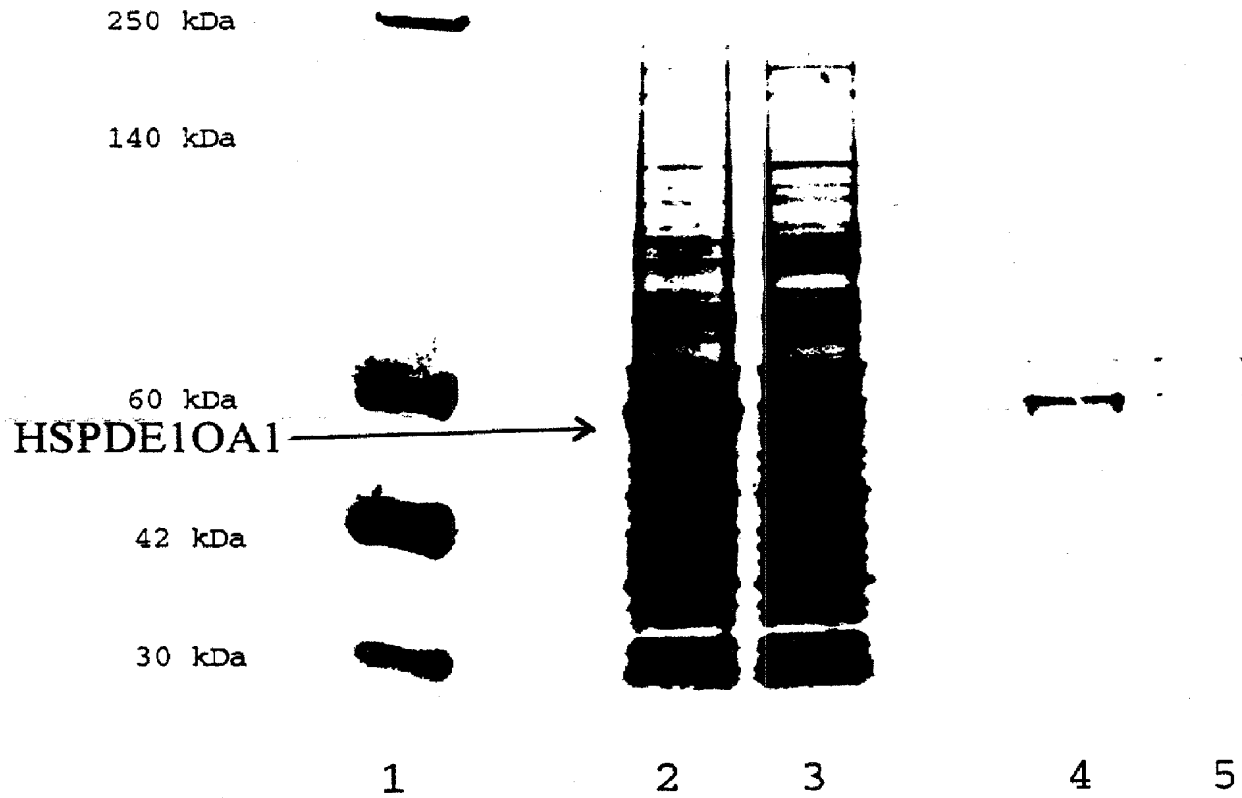


FIGURE 6